

## BREIF COMMUNICATION

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# Effect of Progesterone on Expression of MMP7 and MMP13 in Lungs of Female Mice

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## ABSTRACT

Gender medicine is a new era of science which focuses on the impact of sex hormones and gender on normal physiology, pathobiology and clinical features of diseases. In this study we investigated the impact of pregnancy doses of progesterone hormone on the expression of a couple of matrix metalloproteinase (MMPs), which are known to be involved in tissue remodeling of lungs in health and disease, namely MMP7 and 13.

Pregnancy maintenance dose of progesterone was administered to female BALB/c mice for 21 and 28 days, the control group received PBS for the same days. After removal of the lungs and RNA extraction, quantitative real-time PCR was done using specific primers for MMP7 and MMP13.

We found that progesterone can slightly (not significantly) decrease the expression of MMP13 but had no effect on MMP7.

Our results shows that progesterone has minimal effect on the expression of matrix metalloproteinase7 and matrix metalloproteinase 13, but it may still have an effect on corresponding tissue inhibitor of matrix metalloproteinases (TIMPs) or other components of the Extracellular matrix which remains to be elucidated. Also, the effect of progesterone on these MMPs can be further studied in a fibrosis model.

**Keywords:** Collagenases; Extracellular matrix; Lung diseases; Matrilysin; Matrix metalloproteinases; Progesterone

## INTRODUCTION

Gender medicine is a new era of science which focuses on the effect of different genders and sex

hormones on normal physiology, pathophysiology and clinical features of diseases. While most of the studies on hormonal differences between males and females have been done on disease conditions so far,

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the importance of considering hormonal differences and also hormonal fluctuations in health, especially in normal physiological conditions like puberty, pregnancy and menopause, must be taken into account. In this regard, some studies evaluated the functional parameters of lung in both sexes and during different stages of life.<sup>1</sup> Despite the epidemiological studies on humans, applying mice as available tools to investigate these differences would be a convenient way to shed light on our knowledge in this field. The role of estrogen and progesterone receptors are known in sexual development, but their effect beyond the reproductive system has become an interesting field of study. The Progesterone receptor is not only expressed in reproductive tract but also in mammary glands, cardiovascular system, brain and lungs. Studies with ovariectomized mice showed less hyperresponsiveness to allergens in comparison to normal mice,<sup>2</sup> hence it seems that sex hormones including progesterone can have an effect on physiology of lung function.<sup>3</sup> Matrix metalloproteinases (MMPs) are a family of zinc dependent metalloproteinases with at least 24 members in mammals. They are involved in organogenesis of lungs but their expression is decreased after generation of alveoli.<sup>4</sup> Most studies have evaluated the roles of MMP2 and MMP9 which degrade Gelatin (Collagen IV) but the role of other MMPs such as MMP7 (Matrilysin) and MMP 13 (Collagenase) is poorly understood. MMP7 is constitutively expressed at low levels in epithelial cells lining peribronchial glands, conducting airways and also is produced by macrophages.<sup>5-7</sup> In mice lacking MMP7, the inflammation related to asthma was significantly decreased.<sup>6</sup> MMP13 is expressed by alveolar macrophages and type II pneumocystis, it has a role in lung tissue remodeling and is upregulated in COPD, allergic rhinitis and influenza infection.<sup>8</sup> Moreover, expression of MMP13 is positively controlled by IL-1 and IL-6 and negatively by IL-13.<sup>9-11</sup> Regarding the importance of matrix metalloproteinases in the lung diseases, we aimed to investigate the level of MMP7 and MMP13 in lungs of healthy female mice treated daily with pregnancy maintaining dose of progesterone to evaluate the effect of this sex hormone on those MMPs in the lungs.

## MATERIALS AND METHODS

BALB/c virgin female mice 6-8 weeks old were purchased from Pasteur Institute of Iran and kept at

Center of experimental and comparative studies. All experimental protocols complied with requirements of animal care committee of Iran University of medical sciences (IR.IUMS.REC135.9313675001).

In this study progesterone was injected for 3 weeks equivalent with pregnancy period of mice (19-21 days) at supra physiological concentration similar to pregnancy saving dose in mouse;<sup>12</sup> and also one week extra time (28 days) for researching the effect of toxic doses of progesterone which may occur in progesterone treatment states and autoimmune disorders too.

Mice were randomly divided into three groups (5 mice per group) and experiment was repeated twice; Group1: received Progesterone for 21 days (equal to mouse pregnancy period which is between 19 and 21 days), Group 2: received mouse progesterone for 28 days (an extra week later than pregnancy period to study the progesterone administration consequences) and group 3 mice received daily PBS as control.

The dose of Progesterone used in this study was 1 mg/mouse/day, which is the required dose for maintenance of pregnancy.<sup>12</sup> Progesterone was administered subcutaneously.

Mice were anesthetized with 100-150  $\mu$ L of ketamine and Xylazine mixture (80-100 mg/kg xylazine and 10-12.5mg/kg Ketamine), lungs were removed and homogenized. Total RNA was extracted using "RNX-Plus" (Sinaclon, Iran) according to manufacturer's protocols and was digested with Recombinant Dnase1 (TAKARA BIOTECHNOLOGY). Complementary DNA (cDNA) was synthesized using PrimeScript RT reagent Kit (Takara, Japan) according to manufacturer's instructions.

Gene expression was assessed using SYBR-Green based real-time PCR; the gene specific primers listed in Table 1 were used, and the  $\beta$ -glucuronidase gene was also used to normalize the qPCR data.

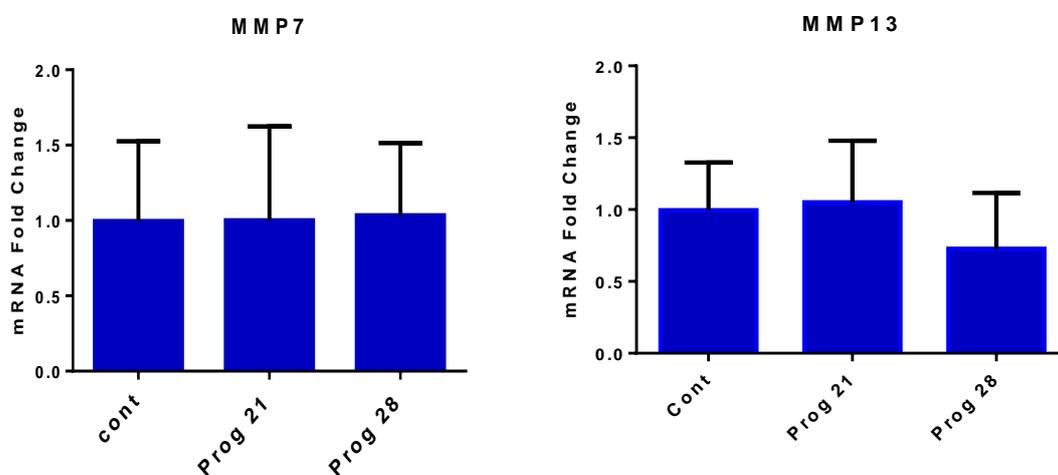
The PCR assay was performed using (Rotor Gene-Q, Germany). All reactions were carried out in duplicate, each reaction contained SYBR Green Master Mix (Takara, Japan) and other components were added according to manufacturer's protocol. Specificity of the primers was determined by melting curve analysis and agarose gel electrophoresis. Amplification conditions were done according to manufacture instructions, the comparative threshold cycle (CT) method (using formula  $2^{-\Delta\Delta CT}$ ) was employed to analyze the obtained data and  $p$  value<0.05 was considered as statistically significant.

## Effect of Progesterone on Expression of MMP7 and MMP13

**Table 1 Sequences used for primer design for evaluation the effect of progesterone on expression of MMP7 and MMP13 in lungs of female mice design**

Primers	Sequences (5' to 3')
(MMP13)	Fw: CAGTTGACAGGCTCCGAGAA Re: TTCACCCACATCAGGCACTC
MMP7	Fw: TGAATTTGGCCACTCTCTGGGTCT Re: TCTGAATGCCTGCAATGTCGTCCT
$\beta$ -glucuronidase	Fw: TGCCTCTGAAGTTAGCCGTG Re: AGTTCTCCGCCGCTTTTA

MMP: matrix metalloproteinase



**Figure 1. mRNA fold change measured by qRT-PCR for evaluation the effect of progesterone on expression of matrix metalloproteinase (MMP) 7 and MMP13 in lungs of normal female mice. Quantitative Real Time PCR of MMP7 and MMP13 in lungs of female mice treated with progesterone, normalized and compared to the housekeeping gene, Beta glucuronidase (GUSB). RNAs were extracted from lungs of mice which received 1 mg daily progesterone for 21 (Prog21) and 28 days (Prog.28) and also mice which received PBS for 28 days (Cont.). No significant fold changes was observed.**

### RESULTS

In order to evaluate the specificity of primers and the absence of primer dimer the amplicon of MMP7, MMP13 and housekeeping gene (Glucuronidase Beta, GUSB) were visualized by electrophoresis.

We found that daily injection of progesterone for 21 and 28 days (pregnancy maintenance dose) did not change the expression level of MMP7 ( $0.9967 \pm 0.2536$  vs  $0.9950 \pm 0.2652$ ,  $p$ -value=0.99 for day 21;  $1.035 \pm 0.1958$  vs  $0.99950 \pm 0.2652$ ,  $p$ -value=0.90 for day 28), but administration of 1mg of progesterone for 28 days slightly decreased the expression of MMP13 in a non-significant manner ( $0.83 \pm 0.17$  vs  $0.99 \pm 0.17$   $p$ -

value=0.54 for day 28;  $1.14 \pm 0.16$  vs  $0.99 \pm 0.17$  for day 21,  $p$ -value=0.60) (Figure 1).

### DISCUSSION

In this in vivo study, after administration of pregnancy maintaining dose of progesterone, the expression of MMP7 and MMP13 was evaluated via qPCR, our results showed that administration of daily progesterone did not affect the expression of MMP7 but slightly decreased the expression level of MMP13 in lungs of healthy female mice in a non-significant manner.

Sex differences and sex hormones apparently affect

lung physiology throughout the life span of human. Both clinical and basic studies have examined sex differences in lung structure and function, in health and disease.<sup>1</sup> Until now to our best knowledge there is no study evaluating the effect of progesterone on healthy mice. In a study done by Lubov Novikova et al, it was observed that interstitial lung disease was worsened by pregnancy;<sup>13</sup> accordingly, in the present study we administered mice with pregnancy maintaining dose of progesterone. Also, there are other conditions in which the pregnancy can induce or exacerbate lung disorders; this includes pulmonary edema, pulmonary embolism, obstructive lung diseases, restrictive lung disease and lung infections.<sup>14</sup> Furthermore, it was shown that estrogen can inhibit TGF- $\beta$  signaling and downstream fibrosis through inhibition of ROS production, whereas progesterone can induce fibrogenic responses through ROS production and hepatic stellate cells activation in hepatic fibrosis.<sup>15</sup> However to our knowledge there is no study addressing the effect of progesterone on normal physiology of lung. Several studies have indicated the regulatory effect of estrogen hormone on hemostasis of extra cellular matrix (ECM) through the increment of MMP2 and MMP9 in mesangial cells.<sup>16</sup> Nevertheless the effect of progesterone was not studied in ECM of lungs. It has been shown that estrogen has a beneficial effect on wound healing through decreasing the expression of MMP8 and increasing collagen synthesis, nevertheless estrogen had no effect on MMP13 expression;<sup>17</sup> which is consistent with our results that progesterone also had no effect on the expression of MMP13. It seems that the expression of MMP13 is under control of some cytokines, and IL-13 as an asthma related cytokine, decreases its expression. Furthermore, TGF- $\beta$  which is one of the main growth factors involved in tissue remodeling, asthma and fibrosis, has been shown to increase the MMP13 expression in fibroblasts.<sup>18</sup> Expression of MMP7 is correlated with lung cancer and has been introduced as a blood biomarker in patients with idiopathic pulmonary fibrosis;<sup>19</sup> so we also decided to evaluate the effect of progesterone on MMP7. Whereas women experience dramatic changes in their sex hormones during different stages of their life, there is paucity of information regarding the effect of progesterone on ECM of lungs tissue; it is not known how progesterone alters tissue repair and remodeling in lungs, therefore based on scant data on this hormone, we decided to explore the effect of this hormone on expression level

of MMP7 and MMP13, which are expressed in epithelial cells lining peribronchial glands, conducting airways and alveolar macrophages.<sup>20</sup> In the present study it was shown that progesterone almost had no effect on MMP7 expression; but on the day 28, progesterone slightly decreased the expression of MMP13, which could implicate that longer exposure to progesterone such as mammals with longer gestation time, or treatment of patients with this hormone might affect MMP13 expression. By the way, increasing the animal numbers per group should be employed to achieve more significant results. Since some studies have indicated that estrogen could influence the expression of some MMPs in diseases, for future work we will also study the effect of progesterone on animal models of diseases related to fibrosis. Accordingly we plan to study the effect of progesterone on MMP7 and MMP13 in a mouse model of scleroderma. We hypothesize that progesterone may also influence other MMPs or their corresponding tissue inhibitor of metalloproteinases (TIMPs). Therefore, to find the missing part of the puzzle regarding the effect of sex hormones on lung tissue remodeling in health, these two MMPs were excluded and we have to focus on other components involved in tissue remodeling. To conclude, progesterone did not change the expression of MMP7 and MMP13 significantly in lungs of normal female mice.

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